

WHAT IS CLAIMED IS:

1. A method of producing a biosensor protein capable of regulating a fluorescence property of green fluorescent protein or its derivative by modifying the structure of green fluorescent protein or its derivative, comprising the steps of:

(A) predicting a hotspot amino acid residue affecting a fluorescence property of green fluorescent protein or its derivative;

10 (B) producing various fusion proteins which have the structure linked with a modified fluorescent protein and one or more functional molecules,

the modified fluorescent protein being the protein obtained by cleaving amino acid sequence of green fluorescent protein or its derivative in the vicinity of the hotspot amino acid residue and modifying the structure of green fluorescent protein or its derivative, and

20 the functional molecules each being the molecules capable of transmitting their conformational changes to the modified fluorescent protein to cause a conformational change of the modified fluorescent protein, thereby altering the fluorescence property of the modified fluorescent protein;

25 (C) reacting the resultant various fusion proteins with a factor affecting the conformation of any of the functional molecules; and

(D) screening a fusion protein exhibiting a change in the fluorescence property by the reaction of the step (C), as a biosensor protein, from the various fusion proteins.

5 2. The method according to claim 1, wherein the fluorescence property is fluorescence intensity.

3. A biosensor protein comprising (1) and (2) below:

10 (1) a modified fluorescent protein which is obtained by cleaving amino acid sequence of green fluorescent protein or its derivative in the vicinity of a hotspot amino acid residue which affects the fluorescence property, and modifying the structure of the green fluorescent protein or its derivative; and

15 (2) one or more functional molecules which are capable of transmitting their conformational changes to the modified fluorescent protein to cause a conformational change of the modified fluorescent protein, thereby altering the fluorescence property of the modified fluorescent protein.

20 4. The biosensor protein according to claim 3, wherein the fluorescence property is fluorescence intensity.

25 5. The biosensor protein according to claim 3, wherein the hotspot amino acid residue corresponds to the 148th amino acid of amino acid sequence of green fluorescent protein.

6. The biosensor protein according to claim 4,
wherein the hotspot amino acid residue corresponds to
the 148th amino acid of amino acid sequence of green
fluorescent protein.

5 7. The biosensor protein according to claim 3,
wherein the hotspot amino acid residue corresponds to
the 94th amino acid of amino acid sequence of green
fluorescent protein.

10 8. The biosensor protein according to claim 4,
wherein the hotspot amino acid residue corresponds to
the 94th amino acid of amino acid sequence of green
fluorescent protein.

15 9. The biosensor protein according to claim 3,
wherein the hotspot amino acid residue corresponds to
the 96th amino acid of amino acid sequence of green
fluorescent protein.

20 10. The biosensor protein according to claim 4,
wherein the hotspot amino acid residue corresponds to
the 96th amino acid of amino acid sequence of green
fluorescent protein.

11. The biosensor protein according to claim 3,
wherein the hotspot amino acid residue corresponds to
the 222nd amino acid of amino acid sequence of green
fluorescent protein.

25 12. The biosensor protein according to claim 4,
wherein the hotspot amino acid residue corresponds to
the 222nd amino acid of amino acid sequence of green

fluorescent protein.

13. A biosensor protein comprising (1) and (2) below:

(1) a modified green fluorescent protein having
5 the following amino acid sequences (a) and (b) in this
order from the N terminus:

(a) an amino acid sequence of X-th to 238th
position of green fluorescent protein; and

10 (b) an amino acid sequence of 1st to Y-th position
of green fluorescent protein,

(where X is an arbitrary number from 148 to 150,
and Y is an arbitrary number from 140 to 147); and

15 (2) one or more functional molecules which are
capable of transmitting their conformational changes to
the modified green fluorescent protein to cause a
conformational change of the modified green fluorescent
protein, thereby altering the fluorescence property of
the modified green fluorescent protein.

14. A biosensor protein comprising the following
20 sequences (a) to (h) sequentially from the N terminus:

(a) an amino acid sequence containing methionine
(linker X);

(b) myosin light chain kinase protein or a partial
amino acid sequence thereof;

25 (c) an amino acid sequence (linker Y) for linking
the above sequence (b) and the following sequence (d);

(d) an amino acid sequence from X-th to 238th

position of green fluorescent protein (where X is an arbitrary number from 148 to 150);

(e) an amino acid sequence for linking the above sequence (d) and the following sequence (f);

5 (f) an amino acid sequence from 1st to Y-th position of green fluorescent protein (where Y is an arbitrary number from 140 to 147);

(g) an amino acid sequence (linker Z) for linking the above sequence (f) and the following sequence (h);

10 and

(h) calmodulin protein or a partial amino acid sequence thereof.

15. A biosensor protein comprising (1) and (2) below:

15 (1) a modified green fluorescent protein having the following amino acid sequences (a) and (b) in this order from the N terminus:

(a) the amino acid sequence from the 149th to 238th position of green fluorescent protein, and

20 (b) the amino acid sequence from the 1st to 144th position of green fluorescent protein; and

(2) one or more functional molecules which are capable of transmitting their conformational changes to the modified green fluorescent protein to cause a conformational change of the modified green fluorescent protein, thereby altering the fluorescence property of the modified green fluorescent protein.

16. A biosensor protein comprising the following sequences (a) to (h) sequentially from the N terminus:

- (a) an amino acid sequence containing methionine (linker X);
- 5 (b) myosin light chain kinase protein or a partial amino acid sequence thereof;
- (c) an amino acid sequence (linker Y) for linking the above sequence (b) and the following sequence (d);
- 10 (d) the amino acid sequence from the 149th to 238th position of green fluorescent protein;
- (e) an amino acid sequence for linking the above sequence (d) and the following sequence (f);
- (f) the amino acid sequence from the 1st to 144th position of green fluorescent protein;
- 15 (g) an amino acid sequence (linker Z) for linking the above sequence (f) and the following sequence (h); and
- (h) calmodulin protein or a partial amino acid sequence thereof.

20 17. The biosensor protein according to claim 3, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

25 18. The biosensor protein according to claim 4, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin

light chain kinase protein or a partial amino acid sequence thereof.

19. The biosensor protein according to claim 5,
wherein the functional molecules are calmodulin protein
5 or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

10 20. The biosensor protein according to claim 6,
wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

15 21. The biosensor protein according to claim 7,
wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

20 22. The biosensor protein according to claim 8,
wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

25 23. The biosensor protein according to claim 9,
wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

24. The biosensor protein according to claim 10,
wherein the functional molecules are calmodulin protein
or a partial amino acid sequence thereof, and myosin
light chain kinase protein or a partial amino acid
sequence thereof.

5 25. The biosensor protein according to claim 11,
wherein the functional molecules are calmodulin protein
or a partial amino acid sequence thereof, and myosin
light chain kinase protein or a partial amino acid
sequence thereof.

10 26. The biosensor protein according to claim 12,
wherein the functional molecules are calmodulin protein
or a partial amino acid sequence thereof, and myosin
light chain kinase protein or a partial amino acid
sequence thereof.

15 27. The biosensor protein according to claim 13,
wherein the functional molecules are calmodulin protein
or a partial amino acid sequence thereof, and myosin
light chain kinase protein or a partial amino acid
sequence thereof.

20 28. The biosensor protein according to claim 15,
wherein the functional molecules are calmodulin protein
or a partial amino acid sequence thereof, and myosin
light chain kinase protein or a partial amino acid
sequence thereof.

25 29. A biosensor protein comprising the following
sequences (a) to (h) sequentially from the N terminus:

- (a) Met-Gly-Thr or Met-Val-Asp (linker X);
(b) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser);
5 (c) Leu-Glu (linker Y);
(d) the amino acid sequence from the 149th to 238th position of green fluorescent protein;
(e) Gly-Gly-Thr-Gly-Gly-Ser (linker amino acid sequence);
10 (f) the amino acid sequence from the 1st to 144th position of green fluorescent protein;
(g) Gly-Thr-Arg or Thr-Arg (linker Z); and
(h) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein.
15 30. A biosensor protein comprising the following sequences (a) to (c) in this order from the N terminus:
(a) the amino acid sequence from the 1st to 144th position of green fluorescent protein;
(b) one or more functional molecules which are capable of transmitting their conformational changes to the modified green fluorescent protein to cause a conformational change of the modified green fluorescent protein, thereby altering the fluorescence property of the modified green fluorescent protein; and
20 (c) the amino acid sequence from the 149th to 238th position of green fluorescent protein.
25 31. The biosensor protein according to claim 30,

wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

5 32. A biosensor protein comprising the following sequences (a) to (g) sequentially from the N terminus:

(a) the amino acid sequence from the 1st to 144th position of green fluorescent protein;

10 (b) an amino acid sequence (linker A) for linking the above sequence (a) and the following sequence (c);

(c) calmodulin protein or a partial amino acid sequence thereof;

(d) an amino acid sequence (linker B) for linking the above sequence (c) and the following sequence (e);

15 (e) myosin light chain kinase protein or a partial amino acid sequence thereof;

(f) an amino acid sequence (linker C) for linking the above sequence (e) and the following sequence (g); and

20 (g) the amino acid sequence from the 149th to 238th position of green fluorescent protein.

33. A biosensor protein comprising the following sequences (a) to (g) sequentially from the N terminus:

25 (a) the amino acid sequence from the 1st to 144th position of green fluorescent protein;

(b) Gly-Thr-Arg (linker A);

(c) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein;

(d) Gly-Thr or Gly-Thr-Gly-Ser-Gly-Gly-Gly-Ser
(linker B);

5 (e) a partial amino acid sequence of myosin light
chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-
Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser);

(f) Thr-Ser (linker C); and

238th position of green fluorescent protein.

34. A biosensor gene encoding the biosensor
10 protein according to claim 3.

35. A biosensor gene encoding the biosensor
protein according to claim 4.

36. A biosensor gene encoding the biosensor
protein according to claim 5.

15 37. A biosensor gene encoding the biosensor
protein according to claim 6.

38. A biosensor gene encoding the biosensor
protein according to claim 7.

20 39. A biosensor gene encoding the biosensor
protein according to claim 8.

40. A biosensor gene encoding the biosensor
protein according to claim 9.

41. A biosensor gene encoding the biosensor
protein according to claim 10.

25 42. A biosensor gene encoding the biosensor
protein according to claim 11.

43. A biosensor gene encoding the biosensor
protein according to claim 12.

44. A biosensor gene encoding the biosensor protein according to claim 13.

45. A biosensor gene encoding the biosensor protein according to claim 14.

5 46. A biosensor gene encoding the biosensor protein according to claim 15.

47. A biosensor gene encoding the biosensor protein according to claim 16.

10 48. A biosensor gene encoding the biosensor protein according to claim 17.

49. A biosensor gene encoding the biosensor protein according to claim 18.

50. A biosensor gene encoding the biosensor protein according to claim 19.

15 51. A biosensor gene encoding the biosensor protein according to claim 20.

52. A biosensor gene encoding the biosensor protein according to claim 21.

20 53. A biosensor gene encoding the biosensor protein according to claim 22.

54. A biosensor gene encoding the biosensor protein according to claim 23.

55. A biosensor gene encoding the biosensor protein according to claim 24.

25 56. A biosensor gene encoding the biosensor protein according to claim 25.

57. A biosensor gene encoding the biosensor

protein according to claim 26.

58. A biosensor gene encoding the biosensor protein according to claim 27.

59. A biosensor gene encoding the biosensor protein according to claim 28.

60. A biosensor gene encoding the biosensor protein according to claim 29.

61. A biosensor gene encoding the biosensor protein according to claim 30.

10 62. A biosensor gene encoding the biosensor protein according to claim 31.

63. A biosensor gene encoding the biosensor protein according to claim 32.

15 64. A biosensor gene encoding the biosensor protein according to claim 33.